REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested. Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version With Markings to Show Changes Made.

In accordance with the Office Action dated January 30, 2001 (copy enclosed), which was issued in connection with the above-identified application, applicants have enclosed the following:

a computer readable form (CRF) copy of the "Sequence Listing," in the form of a 3.5" diskette;

a paper copy of the "Sequence Listing," as well as the above Amendment directing its entry into the specification; and

a statement that the content of the paper and computer readable form are the same and include no new matter.

Support for the amendment to the specification at page 6, lines 12-13 is found in the originally filed Figure 5.

Support for the amendment to the specification at page 13, line 31 to page 14, line 5 is found in Table II, at page 17 of the originally filed application where the pentapeptide sequence is described.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: February 28, 2001

Georgia Evans
Registration No. 44,597

NIXON PEABODY LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603
Telephone: (716) 263-1672
Facsimile: (716) 263-1600

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date below.

2/28/01
Date

Jane C. Wirazyla

Appendix A
Version With Markings to Show Changes Made

In reference to the amendments made herein to the specification, additions appear as underlined text, while deletions appear as bracketed text, as indicated below.

In the Specification:

At page 6, lines 12-13:

Figure 5 demonstrates the binding interactions of src substrate [Ac-Ile-Tyr-Glu-Phe-NH_2] Ac-Ile-Tyr-Gly-Glu-Phe-NH_2 (SEQ. ID. No. 1) in model src active site.

At page 13, line 31 to page 14, line 5:

The standard pentapeptide sequence chosen for the majority of PKA inhibitors in Table 1 was derived from the pseudosubstrate sequence of the peptide inhibitor which was bound to PKA, when the crystal structure illustrated in Figure 1 was solved. The standard pentapeptide sequence used for src in Table 2, [Ac-Ile-AA-Gly-Glu-Phe-NH_2] Ac-Ile-Xaa-Gly-Glu-Phe-NH_2 (SEQ. ID. No. 2), was described in Nair, Kim et al., 1995. Some of the chemistry used to prepare the PKA inhibitors is described in Nair, Lee & Hangauer 1995. The synthetic methodology used to [develope] develop a number of the src inhibitors is described in Lai et al., 1998.

At page 19, line 10 to page 20, line 5:

While testing these boronic acid-containing PKA inhibitors, the corresponding pentapeptide pseudosubstrate inhibitor **20** was included as an internal control while investigating time-dependent inhibition as shown in Table 3. Under Literature Mimetic assay conditions, and no preincubation, the initial results suggested that the shortest chain L-amino acid **21** was binding with the same affinity as the pseudosubstrate inhibitor **20** (i.e. K_i ca. 9 μM). As this side chain was increased in length (to **23** and then **24**) binding affinity appeared to decrease. When the stereochemistry of the unnatural amino acid was inverted from L in **21** to D in **22**, binding affinity appeared to increase 3-fold. This improvement in binding may occur as a result that the boronic acid OH in **21** is positioned at the same chain length as L-homoserine whereas the natural substrate, L-serine, has a one carbon shorter side chain. Modeling results with the PKA ternary structure indicated that the boronic acid OH can be

retracted back somewhat by inverting the α-carbon stereochemistry from L in **21** to D in **22** and then repositioning the side chain to more closely mimic the positioning of the natural substrate L-serine OH adjacent to the catalytic residues (Asp-166 and Arg-168). The modeling results were subsequently supported by the finding that, upon incubation of PKA with these inhibitors for up to 4 hours without adding the competing peptide substrate (Kemptamide: LRRASLG-NH_2) (SEQ. ID. No. 5), both 21 and 22 function as substrates with the D-diastereomer 22 being phosphorylated faster.

At page 22, line 13 to page 23, line 4:

Since the src and IRTK structures are only used as qualitative guides in designing the non-peptide scaffolds and combinatorial libraries, the active sites along with two layers of surrounding residues were carved out from the native src and IRTK ternary structures, analogous to the previous PKA modeling studies. The IRTK:peptide:AMP-PNP ternary structure active site region was used as the template structure to guide the building of the src residue sequence 424-418 back onto the src structure using the comparative homology modeling technique (see Hutchins & Greer, 1991). These residues were disordered in the native src crystal structure and therefore not visible by x-ray. They were reintroduced because they help form the P+1 to P+3 binding sites for peptide substrates which are important for some of the modeling studies. The analogous residues in the IRTK ternary structure are seen by x-ray and directly interact with the bound peptide substrate. In fact, it is probably the presence of the bound peptide substrate which induces order in the positioning of this sequence so that it is visible by x-ray. The src pentapeptide substrate Ac-Ile-Tyr-Gly-Glu-Phe-NH_2 (SEQ. ID No. 1) (Nair et al., 1995) was then docked into the src active site again using the IRTK ternary structure as a template. Small adjustments were then manually made to partially clean up this complex, all of the hydrogen atoms were added, appropriate formal and partial charges (calculated via the Gasteiger Marsili method) were added, and then the entire complex was subjected to 300 iterations of molecular mechanics minimization using the Tripos force field, [analgous] analogous to the previous PKA modeling procedure. A schematic representation of this modeled complex is given in Figure 5. Any inaccuracies in this src:peptide and the src:inhibitor models are accommodated by experimentally evaluating a range of side chains, the number and diversity of which is scaled roughly to the level of uncertainty for the structure of their particular binding region in the src model active site (see later), in a combinatorial fashion.